

**IN THE CLAIMS**

This listing of claims replaces all prior versions, and listings, in this application.

1. (previously presented) A method for determining the amount of template nucleic acid present in a sample comprising:

- i) bringing into association with the sample all the components necessary for nucleic acid amplification, and all the components necessary for a bioluminescence assay for nucleic acid amplification including:
  - a) a nucleic acid polymerase,
  - b) the substrates for the nucleic acid polymerase,
  - c) at least two primers,
  - d) a thermostable luciferase,
  - e) luciferin,
  - f) ATP sulphurylase, and
  - g) adenosine 5' phosphosulphate;

and subsequently:

- ii) performing a nucleic acid amplification reaction of the template nucleic acid involving more than one cycle of amplification;
- iii) monitoring the intensity of light output from the bioluminescence assay; and
- iv) determining the amount of template nucleic acid present in the sample.

2. (previously presented) A method according to claim 1, wherein at least ii) and iii) are carried out in a sealed vessel.

3. (previously presented) A method according to claim 1, wherein in iii) the intensity of light output is monitored during the nucleic acid amplification reaction.

4. (previously presented) A method according to claim 1, wherein iii) further includes producing a data set of intensity of light output as a function of time.

5. (previously presented) A method according to claim 4, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of change of intensity of light output changes significantly.
6. (currently amended) A method according to claim 1, wherein the amount of template nucleic acid present in the sample ~~is determined~~ before the nucleic acid amplification reaction of ii) is determined.
7. (currently amended) A method according to claim 1, wherein the amount of template nucleic acid present in the sample ~~is determined~~ after the nucleic acid amplification reaction of ii) is determined.
8. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output begins to increase.
9. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output is at a maximum.
10. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output increases.
11. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the rate of decrease of intensity of light output decreases.

12. (previously presented) A method according to claim 5, wherein the amount of template nucleic acid present is determined by measuring from the data set the time taken to reach a point at which the intensity of light output reaches or crosses a predetermined level.

13. (previously presented) A method according to claim 8, wherein the thermostable luciferase that is brought into association with the sample in i) is a reversibly-inhibited luciferase.

14. (previously presented) A method according to claim 1, wherein iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which the sample comprises a known amount of template nucleic acid.

15. (previously presented) A method according to claim 1 for determining whether the template nucleic acid is present in the sample, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data set whether the intensity of light output reaches or crosses a predetermined level.

Claim 16 (canceled)

17. (original) A method according to claim 15, wherein an increase in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

18. (original) A method according to claim 15, wherein a decrease in the intensity of light output relative to the predetermined level indicates the presence of template nucleic acid in the sample.

19. (previously presented) A method according to claim 15, wherein whether the template nucleic acid is present in the sample is determined by measuring from the data

set whether the intensity of light output reaches or crosses the predetermined level within a predetermined length of time following the start of the amplification reaction of ii).

20. (previously presented) A method according to claim 1, wherein iv) further comprises comparing the intensity of light output to the intensity of light output from a control in which no amplification has taken place.

21. (original) A method according to claim 20, wherein a decrease in the intensity of light output relative to a control reaction in which no amplification has taken place indicates the presence of template nucleic acid in the sample.

22. (previously presented) A method according to claim 1, wherein the nucleic acid amplification reaction of ii) is a low temperature thermocycling amplification method in which the cycling temperature range does not exceed 75°C.

23. (previously presented) A method according to claim 1, wherein the nucleic acid amplification reaction of ii) is carried out isothermally.

24. (previously presented) A method according to claim 23, wherein the nucleic acid amplification reaction of ii) is carried out within a temperature range that does not exceed 75°C.

25. (previously presented) A method according to claim 23, wherein the nucleic acid amplification reaction of ii) is carried out at a constant temperature at which the components of the amplification reaction and the bioluminescence assay are stable.

26. (previously presented) A method according to claim 23, wherein the nucleic acid amplification reaction of ii) is carried out at more than one temperature within the

temperature range in which the components of the amplification reaction and the bioluminescence assay are stable.

27. (previously presented) A method according to claim 26, wherein the nucleic acid amplification reaction of ii) is started at a higher temperature and subsequently dropped to a lower temperature.

28. (previously presented) A method according to claim 1 further comprising determining a medical diagnosis.

29. (previously presented) A method according to claim 1 further comprising determining whether a pathogen is present in a sample.

30. (previously presented) A method according to claim 1 further comprising determining whether a particular nucleic acid sequence is present in an organism's genetic code.

31. (previously presented) A method according to claim 30 further comprising determining whether the nucleic acid to which the template nucleic acid originates has been genetically modified.

32. (previously presented) A method according to claim 1 further comprising determining whether an organism is present in a sample.

33. (previously presented) A method according to claim 1, wherein the template nucleic acid is linked to an antibody.

Claims 34-37 (canceled)

38. (previously presented) A method for determining the amount of template nucleic acid present in a sample comprising:

- i) bringing into association with the sample all the components necessary for nucleic acid amplification, and all the components necessary for a bioluminescence assay for nucleic acid amplification including:
  - a) a nucleic acid polymerase,
  - b) the substrates for the nucleic acid polymerase,
  - c) at least two primers,
  - d) a thermostable luciferase, and
  - e) luciferin;

and subsequently:

- ii) performing a nucleic acid amplification reaction of the template nucleic acid involving more than one cycle of amplification;
- iii) monitoring the intensity of light output from the bioluminescence assay; and
- iv) determining the amount of template nucleic acid present in the sample.